

## THE "ACCESSORY CHROMOSOME" IN EPEIRA.

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### INTRODUCTION.

The problem involved in the following paper was suggested to me in August, 1905, by Professor E. B. Wilson, in view of the apparent contradiction that occurs between the insects and spiders in regard to the origin and behavior of the so-called "accessory chromosome."

In many of the Orthoptera (Acrididæ ('00), and Locustidæ ('02) McClung; Acrididæ ('00 and '02) Sutton; and Phasmidæ ('01) de Sinéty) the accessory chromosome has been found to be derived from a single, univalent spermatogonial chromosome, which splits longitudinally during the growth period, the two halves passing bodily to one pole in the first division. In the second division, however, these two halves were found to separate, being distributed equally to each of the resulting spermatids. Professor Wilson in his "Studies on Chromosomes," II. ('05), gives a general account of the accessory chromosome in the Hemiptera, which is consistent with the preceding results in the Orthoptera, though not identical. He says, p. 533: "The 'accessory' or heterotropic chromosome is certainly in most Hemiptera—and I believe will be found in all—unpaired in the spermatogonia, and its behavior is throughout that of a univalent body. . . . This chromosome divides in only one of the maturation divisions, passing undivided to one pole of the spindle in the other. The latter division is usually the second (*Pyrrochoris*, *Anasa*, *Protenor*, etc.), but in *Archimerus* and *Banasa* it is the first. In either case one half the spermatozoa receive one more chromosome than the other half."

Opposed to the foregoing conclusions are those of Montgomery ('05), obtained from a study of *Syrbula* (one of the Acrididæ), and of *Lycosa*, a spider. In these two unrelated forms he found the accessory chromosome of the growth period to be formed by the union of *two* univalent spermatogonial chromo-

somes at the time of synapsis. The mode of division of this bivalent accessory was not positively determined, but he suggested that the similarity in its formation gives some evidence that it may behave in the same way as the other chromosomes in both mitoses, dividing first reductionally and then equationally, thus being equally distributed to all of the spermatids. Nearly at the same time Wallace ('05) published her final paper on the spermatogenesis of *Agalena*, a spider, with results quite different from those of Montgomery on *Lycosa*. I will quote her brief summary, p. 182.

"1. The spermatogonia contain two accessory chromosomes and thirty-eight other chromosomes.

"2. In the primary spermatocytic division, the two accessory chromosomes pass over undivided into one of the daughter cells. The reduced number of other chromosomes is nineteen and these divide transversely.

"3. In the secondary spermatocytic division, the two accessory chromosomes again pass over undivided into one of the daughter cells. The nineteen other chromosomes divide longitudinally.

"4. Only one fourth of the spermatozoa contain the accessory chromosomes.

"5. Apparently the remaining three fourths of the spermatozoa degenerate after almost or altogether reaching maturity. In this respect they are regarded as homologous to the polar bodies thrown off by the ovum."

In view of these perplexing and contradictory accounts, I undertook the present work on *Epeira scolopetaria* to see if I could throw any light on the question. Owing to lack of material I have been able to study only one family, the Epeiridæ. The results which I have to offer on the origin and behavior of the accessory chromosome here are consistent with those of McClung, Sutton and Wilson, already mentioned, and also with those of Blackman on the myriapods ('03), but they give no explanation of the results of Wallace and Montgomery, which are widely different from my own. At a future time I hope to examine other families of spiders with respect to this discrepancy; certainly in none of my preparations of *Epeira* do I find any trace of degenerating spermatozoa.

I am greatly indebted to Professor Wilson for assistance in directing this work, and in the preparation of this paper. I wish also to thank Professor Calkins for his kindness in correcting the manuscript.

#### TERMINOLOGY, METHODS, ETC.

At Professor Wilson's suggestion I shall designate the "accessory chromosome" as the "odd chromosome," a name first used by Montgomery in a somewhat different sense.<sup>1</sup> Since there is no reason for calling it the "accessory chromosome," and since it behaves heterotropically in only one division, it seems advisable to adopt the simpler name, with the important significance that it has no mate throughout the history of the male germ cells.

Material was obtained in August and September, 1905, and also again in the early spring of 1906. The testes were dissected out rapidly in the fixing fluid, instead of the customary normal salt solution, as the process was thus rendered much easier. Strong Flemming gave the best fixation, and the finest results for general study were obtained with iron hæmatoxylin and pure saffranin, the latter giving especially beautiful and valuable results. Several different stains were tried as a differential test for a plasmosome, thionin, Auerbach and Flemming-triple. Long-extracted iron hæmatoxylin slides were studied also, but in no case could I find any trace of a plasmosome.

The figures for this paper are camera drawings made with a compensating ocular, No. 12, and a  $\frac{1}{12}$  oil immersion lens. They were enlarged  $2\frac{1}{2}$  diameters with a drawing camera, corrected from the original, and then reduced one half in the final plates.

#### SPERMATOGONIAL CHROMOSOMES.

Longitudinal sections of the testes give a complete series of stages from the resting spermatogonia around the periphery to the ripe spermatozoa in the central lumen. For this work it did not seem necessary to make a detailed study of the spermatogonia, the one important thing being to determine the number

<sup>1</sup> In his latest paper ('06) Montgomery recognizes the fact that there is no distinction between the "odd chromosome" and the accessory, as was pointed out by Wilson ('05). Montgomery now proposes the term "monosome" for this chromosome.

of spermatogonial chromosomes. With this in view I selected eight of the best polar metaphases of the last spermatogonial division, and drew them carefully with the camera. Seven of the



FIG. 1. *a-c*, last spermatogonial division; *a*, side-view of spindle; *b*, *c*, metaphase groups, showing twenty-three chromosomes, odd chromosome (*o*); *d-i* growth period, showing characteristic forms of the odd chromosome (*o*); *d*, contraction phase, early growth period; *e*, *f*, later stages showing the shortening and thickening of the spireme threads; *g*, *h*, late growth period, beginning of condensation; *i*, prophase of first division.

eight camera drawings gave a count of twenty-three chromosomes. In the eighth the chromosomes were so compact that I could not count definitely beyond nineteen, but from the seven

good ones it seemed conclusive that twenty-three was the correct number. Owing to the fact that the chromosomes often lie one upon the other, these counts were made with difficulty. In Fig. 1, *b* and *c*, distinct size and form differences may be readily observed. These variations are due in part to foreshortening, yet allowing for this error twenty-two of the chromosomes can be symmetrically paired off into eleven pairs, while the twenty-third is left without a mate. This is the small, round chromosome marked *O*. Since this chromosome has no mate, it can take no part in synapsis, and I infer that it is the odd chromosome, which persists as a compact, deeply-staining nucleolus throughout the growth period, Fig. 1, *d-i*.

#### GROWTH PERIOD.

Fig. 1, *d*, represents the contraction phase of the early synaptic period, with the ordinary chromosomes in the form of a closely massed spireme, while the odd chromosome appears as a large compact, deeply-staining nucleolus at one side of the contraction figure. Even as early as this the odd chromosome often appears double as in *f*, consisting of two closely approximated halves. This I interpret as the result of an equation-division. This double nature has been observed in other forms (*cf.* *Syromastes*, Gross; *Anasa*, Wilson; *Brachystola*, Sutton; Acrididae, McClung). In the spiders Wallace and Montgomery have described the two accessory chromosomes as still separated at this period. In my own preparations I find a few cells, of the early growth-period, with two compact nucleoli, sometimes lying side by side and sometimes widely separated. At first I thought one of these might be a plasmosome, but with all of the differential stains mentioned above, both bodies took the nuclear stain equally well. These cells are of rare occurrence, and are, I think, abnormalities, resulting from this early equation-division of the odd chromosome.

In a later stage (Fig. 1, *e*) the chromosome threads loosen, and appear as longitudinally-split rods, with knobbed ends, the odd chromosome here being often bipartite in appearance. These chromosome threads shorten and thicken to form the chromosomes of the late growth period. In *g* and *h* the eleven ordinary

chromosomes have assumed the characteristic forms of rods, V's, rings, crosses, etc., preparatory to the final condensation of stage *i*.

#### MATURATION DIVISIONS.

In Fig. 1, *g* and *i*, the odd chromosome is seen in two of its most characteristic forms, which we encounter again and again

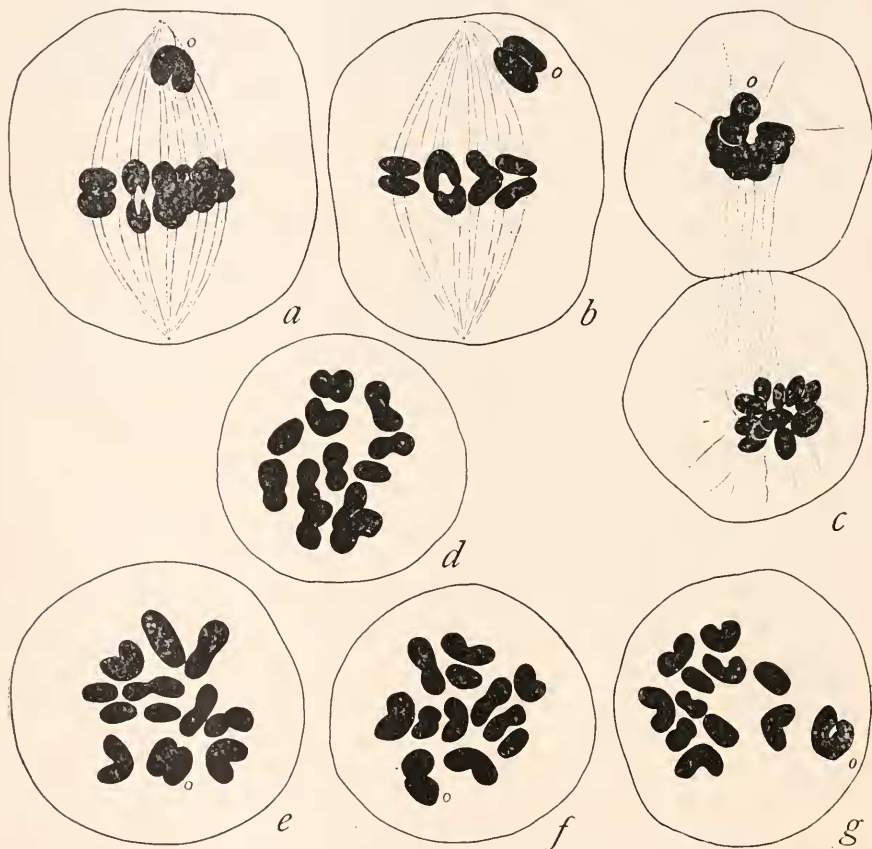


FIG. 2. First spermatocyte-division; *a*, *b*, metaphase-figures, side-view, showing odd chromosome passing undivided to one pole; *c*, telophase showing the odd chromosome present in but one of the daughter-cells; *d*, *e*, *f*, *g*, metaphase-figures, polar view, showing the odd chromosome, identified by its different level.

on the first division spindles. Fig. 2, *a* and *b*, represent such forms, attached by one spindle fiber and passing bodily to one pole in advance of the other chromosomes. These first division



figures are exceedingly numerous, the odd chromosome always being in this relation to the other chromosomes. From the similar shapes and positions of the ordinary chromosomes on the spindle, as compared with those described by other observers, I should say that the first division in *Epeira* is a reduction division and the second an equation-division, but I have not yet studied for this point and have no new evidence to offer.

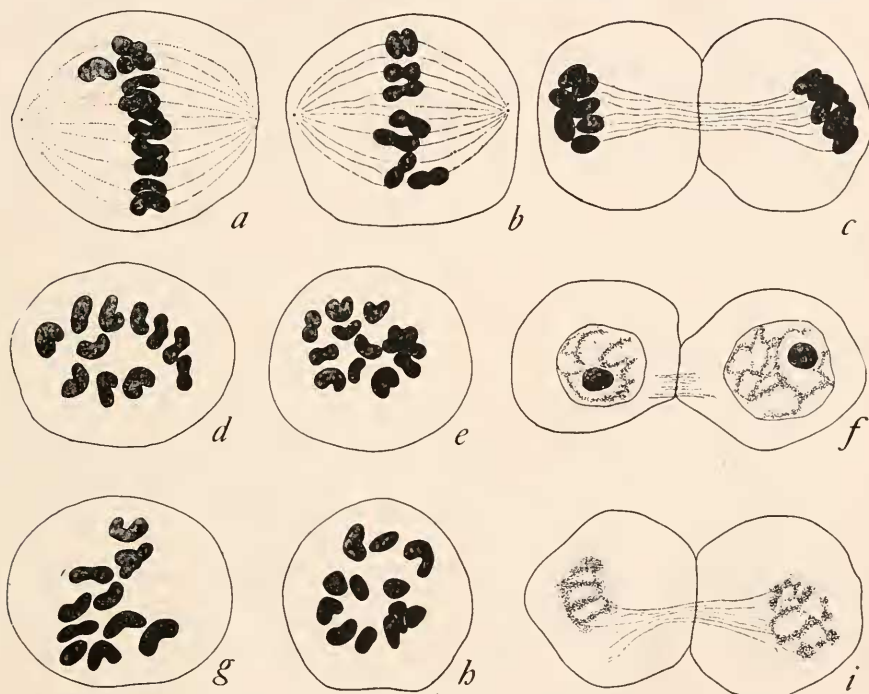


FIG. 3. Second spermatocyte-division; *a*, *b*, spindles, side-view, *c*—late anaphase; all of the chromosomes not pictured in *b* and *c*; *d*, *g*, metaphase-figures, showing eleven chromosomes; *e*, *h*, metaphase-figures, showing twelve chromosomes; *f*, *i*, telophases—*f* showing presence and equal distribution of odd chromosome, *i* showing absence of odd chromosome.

Fig. 2, *d*–*g*, represents first division polar metaphases showing the eleven ordinary chromosomes and the odd chromosome, *O*, which can always be recognized by its different level.

Fig. 2, *c*, represents a first division telophase, showing the odd chromosome in but one of the daughter cells (*o*, in the upper

half). In the lower cell the eleven ordinary chromosomes can be counted. Resulting from the division of each primary spermatocyte are two types of secondary spermatocytes, one containing twelve chromosomes, and one eleven. These two types divide, giving polar metaphase groups that show either twelve or eleven chromosomes (*cf.* Fig. 3, *d* and *g* with eleven, and *e* and *h* with twelve chromosomes).

In side view the second division spindles are all of exactly the same type (Fig. 3, *a* and *b*). I have studied a great many of them, and in no case do I find the odd chromosome passing undivided to one pole as Miss Wallace describes for *Agalena*.<sup>1</sup>

As I have suggested before, the second division is an equational-division, and the odd chromosome divides, in all probability, along its original split, thus being distributed equally to each of the resulting spermatids. The telophase, Fig. 3, *f*, represents two of the early spermatids derived from the preceding type of division, the odd chromosome still recognizable by its definite shape and deep-staining reaction. From the division of the other type of spermatocyte Fig. 3, *i* (without the odd chromosome), are derived two spermatids without this dark nucleolus. There are, therefore, two types of spermatids existing in equal numbers, one half with the odd chromosome, and one half without. To establish this point, I counted in forty-four different fields, all of the early spermatids with the odd chromosome, and all of those without. This count gave a total of 287 of the former and 286 of the latter, thus giving very positive evidence that the two types of spermatids exist in the proportion of half to half.

#### SUMMARY.

1. The spermatogonia contain twenty-three chromosomes, twenty-two ordinary ones and the odd chromosome.
2. The odd chromosome is the chromosome-nucleolus of the growth period.
3. In the first maturation-division the odd-chromosome passes

<sup>1</sup> It seems possible that Miss Wallace may have mistaken a first division spindle for a second-division. Her two types of second-division spindles, Figs. 27 and 28, Plate IX., vary considerably in size, and by comparison of the larger, Fig. 28 (the one with the accessory chromosomes), with Fig. 18, it could, I think, easily represent a first division.



undivided to one pole, the eleven ordinary chromosomes dividing by a reduction-division.

4. The second maturation division is an equation division, in which the odd chromosome participates.

5. Resulting from the first division are two types of secondary spermatocytes (1) containing eleven chromosomes, plus the odd chromosome, and (2) containing only eleven chromosomes.

Resulting from the second division are two types of spermatozoa (1) containing eleven chromosomes, plus the odd chromosome, and (2) containing only eleven chromosomes. These two types of spermatozoa exist in equal numbers.

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